EBV ELISA
IgG Testkit / IgM Testkit

Order No.: EC202.00  Color Coding: yellow/red
EBV EA-D:  

Order No.: EC204.00  Color Coding: yellow/light blue
EBV EBNA1:  

Order No.: EC205G00  Color Coding: yellow/orange
EBV VCA IgG:  

Order No.: EC203M00  Color Coding: yellow/black
EBV VCA IgM:  

FOR IN VITRO DIAGNOSIS ONLY

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1. **Intended Use**

The EBV EA-D, EBV EBNA1, EBV VCA ELISAs are intended for the semiquantitative detection of antibodies against the several markers of Epstein Barr Virus. In combination the kits are intended to differentiate or prove seronegativity, primary infection or past infection.

2. **Diagnostic Relevance**

The Epstein Barr virus belongs to the family of the Herpesviridae and is transmitted mainly by saliva, when it infects the epithelial cells of the oropharynx initially and then the B-lymphocytes. The virus is the cause of infectious mononucleosis (IM) and chronic active EBV infection. There is also an association between EBV infections and Burkitt's lymphoma and also nasopharyngeal carcinomas in Africa and Asia. According to serological investigations, approx. 95% of adults are seropositive for EBV.

Primary EBV infections are normally asymptomatic but can be the cause of infectious mononucleosis in adolescents and young adults. IM is a self-limiting illness and is characterised by lymphadenopathy, fever, hepatosplenomegaly and leukocytosis with atypical lymphocytes (1-7).

The purpose of EBV serology is to differentiate or confirm seronegativity, primary infections together with past infections and the differential diagnostic elimination of infections involving diseases with clinically similar symptoms caused by: CMV, rubella virus, mumps virus, HIV, HAV, HBV, HCV and neurotropic viruses as well as brucellosis, listeriosis, leptospirosis, toxoplasmosis and neoplastic diseases such as lymphoma und leukaemia (8).

3. **Test Principle**

The antibody searched for in the human serum forms an immune complex with the antigen coated on the microtiter-plate. Unbound immunoglobulins are removed by washing processes. The enzyme conjugate attaches to this complex. Unbound conjugate is again removed by washing processes. After adding the substrate solution (TMB), a blue dye is produced by the bound enzyme (peroxidase). The color changes to yellow when the stopping solution is added.

4. **Package Contents**

4.1 **Package contents EBV EA-D, EBV EBNA1, EBV VCA IgG**

1. **1 Microtiter-Plate** consisting of 96 w ith antigen coated, breakable single w ells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7.2, w ith preservative and Tw een 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7.2, w ith preservative and Tw een 20
4. **IgG negative Control, 2000µl**, human serum w ith protein-stabilizer and preservative, ready to use
5. **IgG cut-off Control, 2000µl**, human serum w ith protein-stabilizer and preservative, ready to use
6. **IgG positive Control, 2000µl**, human serum w ith protein-stabilizer and preservative, ready to use
7. **IgG-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with protein-stabilizer and preservative in Tris-Buffer, ready to use
8. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
9. **Citrate-Stopping Solution, 6ml**, contains an acid mixture

4.2 **Package contents EBV VCA IgM**

1. **1 Microtiter-Plate** consisting of 96 w ith antigen coated, breakable single w ells, lyophilised
2. **PBS-Dilution Buffer (blue, ready to use) 2x50ml**, pH 7.2, w ith preservative and Tw een 20
3. **PBS-Washing Solution (20x concentrated) 50ml**, pH 7.2, w ith preservative and Tw een 20
4. **IgM negative Control, 2000µl**, human serum w ith protein-stabilizer and preservative, ready to use
5. **IgM cut-off Control, 2000µl**, human serum w ith protein-stabilizer and preservative, ready to use
6. **IgM positive Control, 2000µl**, human serum w ith protein-stabilizer and preservative, ready to use
7. **IgM-Conjugate (anti-human), 11ml**, (sheep or goat)-horseradish-peroxidase-conjugate with FCS and preservative in Tris-Buffer, ready to use
8. **Tetramethylbenzidine substrate solution (3,3',5,5'-TMB), 11ml**, ready to use
9. **Citrate-Stopping Solution, 6ml**, contains an acid mixture
5. **Storage and Shelflife of the Testkit and the ready to use reagents**

Store the testkit at 2-8°C. The shelf life of all components is shown on each respective label; for the kit shelf life please see Quality Control Certificate.

1. Microtiter strips/single wells are to be resealed in package after taking out single wells and stored with desiccant at 2-8°C. Reagents should immediately be returned to storage at 2-8°C after usage.
2. The ready to use conjugate and the TMB-substrate solution are sensitive to light and have to be stored in dark. Should there be a color reaction of the substrate dilution due to incidence of light, it is not usable anymore.
3. Take out only the amount of ready to use conjugate or TMB needed for the test insertion. Additional conjugate or TMB taken out may not be returned but must be dismissed.

<table>
<thead>
<tr>
<th>Material</th>
<th>Status</th>
<th>Storage</th>
<th>Shelflife</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Samples</td>
<td>Undiluted</td>
<td>+2 to +8°C</td>
<td>max. 6h</td>
</tr>
<tr>
<td>Controls</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>1 week</td>
</tr>
<tr>
<td>Microtitreplate</td>
<td>After Opening</td>
<td>+2 to +8°C (storage in the provided bag with desiccant bag)</td>
<td>3 months</td>
</tr>
<tr>
<td>Rheumatoid factor - Absorbent</td>
<td>Undiluted, After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Conjugate</td>
<td>Diluted</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Tetramethylbenzidine</td>
<td>After Opening</td>
<td>+2 to +8°C (protect from light)</td>
<td>3 months</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>After Opening</td>
<td>+2 to +8°C</td>
<td>3 months</td>
</tr>
<tr>
<td>Final Dilution (ready-to-use)</td>
<td>After Opening</td>
<td>+2 to +25°C</td>
<td>4 weeks</td>
</tr>
</tbody>
</table>

6. **Precautions and Warnings**

1. Only sera which have been tested and found to be negative for HIV-1 antibodies, HIV-2 antibodies, HCV antibodies and Hepatitis-B surface-antigen are used as control sera. Nevertheless, samples, diluted samples, controls, conjugates and microtiter strips should be treated as potentially infectious material. Please handle products in accordance with laboratory directions.
2. Those components that contain preservatives, the Citrate Stopping Solution and the TMB have an irritating effect to skin, eyes and mucous. If body parts are contacted, immediately wash them under flowing water and possibly consult a doctor.
3. The disposal of the used materials has to be done according to the country-specific guidelines.

7. **Material required but not supplied**

1. Aqua dest./demin.
2. Eight-channel pipette 50µl, 100µl
3. Micropipettes: 10µl, 100µl, 1000µl
4. Test tubes
5. Paper towels or absorbent paper
6. Cover for ELISA-plates
7. Disposal box for infectious material
8. ELISA hand washer or automated EIA plate washing device
9. ELISA plate spectrophotometer, wavelength = 450nm, reference length = 620nm (Reference Wavelength 620-690nm)
10. Incubator

8. **Test Procedure**

Working exactly referring to the Sekisui Virotech user manual is the prerequisite for obtaining correct results.

8.1 **Examination Material**

Either serum or plasma can be used as test material, even if only serum is mentioned in the instructions. Any type of anticoagulant can be used for plasma.
Always prepare patient-dilution freshly.
For a longer storage the sera must be frozen. Repeated defrosting should be avoided.

1. Only fresh non-inactivated sera should be used.
2. Hyperlipaemic, haemolytic, microbiobically contaminated and turbid sera should not be used (false positive/negative results).

8.2 Preparation of Reagents

The Sekisui Virotech System Diagnostica offers a high degree of flexibility regarding the possibility to use the dilution buffer, washing solution, TMB, citrate stopping solution as well as the conjugate for all parameters and for all different lots. The ready to use controls (positive control, negative control, cut-off control) are parameter specific and only to use with the plate lot indicated in the Quality Control Certificate.

1. Set incubator to 37°C and check proper temperature setting before start of incubation.
2. Bring all reagents to room temperature before opening package of microtiter strips.
3. Shake all liquid components well before use.
4. Make up the washing solution concentrate to 1 L with distilled or demineralised water. If crystals have formed in the concentrate, please bring the concentrate to room temperature before use and shake well before use.
5. High IgG-titer or rheumatoid factors may disturb the specific detection of IgM-antibodies and may lead to false positive resp. false negative results. For a correct IgM-determination it is therefore necessary to pre-treat the sera with RF-SorboTech (VIROTECH adsorbent). For IgM-controls a pre-absorbent treatment is not necessary.

8.3 Virotech ELISA Test Procedure

1. For each test run, pipette 100µl each of ready to use dilution buffer (blank), IgG- and IgM-positive, negative and cut-off controls as well as diluted patient sera. We propose a double insertion (blank, controls and patient sera); for cut-off control a double insertion is absolutely necessary. Working dilution of patient sera: 1+100; e.g. 10µl serum + 1ml dilution buffer.
2. After pipetting start incubation for 30 min. at 37°C (w ith cover).
3. End incubation period by washing microtiter strips 4 times with 350 – 400µl washing solution per well. Do not leave any washing solution in the wells. Remove residues on a cellulose pad.
4. Pipette 100µl of ready to use conjugate into each well.
5. Incubation of conjugates: 30 min. at 37°C (w ith cover).
6. Stop conjugate incubation by washing 4 times (pls. refer to point 3 above).
7. Pipette 100µl of ready to use TMB into each well.
8. Incubation of substrate solution: 30 min. at 37°C (w ith cover, keep in dark).
9. Stopping of substrate reaction: pipette 50µl of citrate stopping solution into each well. Shake plate carefully and thoroughly until liquid is completely mixed and a homogeneous yellow color is visible.
10. Measure extinction (OD) at 450/620nm (Reference Wavelength 620-690nm). Set your photometer in such a way that the blank value is deducted from all other extinctions. Extinctions should be measured within 1 hour after adding the stopping solution!

Pls. refer to last page for Test Procedure Scheme

8.4 Usage of ELISA processors

All Sekisui Virotech ELISAs can be used on ELISA processors. The user is bound to proceed a validation of the devices (processors) on a regular basis.

Sekisui Virotech recommends the following procedure:

1. Sekisui Virotech recommends to proceed the validation of device referring to the instructions of the device manufacturer during the implementation of the ELISA processor respectively after bigger reparations.
2. It is recommended to check the ELISA-processor with the Validationkit (EC250.00) afterwards. A regular check using the Validationkit shall be proceeded minimum once a quarter to test the accuracy of the processor.
3. The release criteria of the Quality Control Certificate of the product must be fulfilled for each testrun.

With this procedure, your ELISA processor will function properly and this will support quality assurance in your laboratory.
9. Test Evaluation

The ready to use controls serve for a semiquantitative determination of specific IgG- and IgM-antibodies. Their concentration can be expressed in Virotech units = VE. Fluctuations resulting from the test procedure can be balanced with this calculation method and a high reproducibility is achieved in this way. Use the means of the OD values for calculation of the VE.

9.1 Test function control

a) OD-values
The OD of the blank should be < 0.15.
The OD-values of the negative controls should be lower than the OD-values mentioned in the Quality Control Certificate. The OD-values of the positive controls as well as of the cut-off controls should be above the OD-values mentioned in the Quality Control Certificate.

b) Virotech Units (VE)
The Virotech Units (VE) of the cut-off controls are defined as 10 VE. The calculated VE of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

If those requirements (OD-values, VE) are not fulfilled, the test has to be repeated.

9.2 Calculation of the Virotech Units (VE)
The extinction of the blank value (450/620nm) has to be subtracted from all other extinctions.

\[
VE\text{ (positive control)} = \frac{OD\text{ (positive control)}}{OD\text{ (cut-off control)}} \times 10
\]

\[
VE\text{ (patient serum)} = \frac{OD\text{ (patient serum)}}{OD\text{ (cut-off control)}} \times 10
\]

9.3 Interpretation Scheme IgG and IgM

<table>
<thead>
<tr>
<th>Result (VE)</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9,0</td>
<td>negative</td>
</tr>
<tr>
<td>9,0 - 11,0</td>
<td>borderline</td>
</tr>
<tr>
<td>&gt; 11,0</td>
<td>positive</td>
</tr>
</tbody>
</table>

1. If the measured values are above the defined borderline range, they are considered to be positive.
2. If the measured VE is within the borderline range, no significant high antibody concentration is present, the samples are considered to be borderline. For the secure detection of an infection it is necessary to determine the antibody concentration of two serum samples. One sample shall be taken directly at the beginning of the infection and a second sample 5 – 10 days later (convalescent serum). The antibody concentration of both samples has to be tested in parallel, that means in one test run. A correct diagnosis based on the evaluation of a single serum sample is not possible.
3. If the measured values are below the defined borderline range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.
9.4 Meaning of the Antigens

<table>
<thead>
<tr>
<th>Antigen/Description</th>
<th>Meaning of the antigens</th>
<th>Specificity of the antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>EBNA1</td>
<td>Epstein-Barr Nuclear Antigen, a viral protein, which is expressed in the nucleus of latent infected cells. IgG antibodies against EBNA1 are regarded as reliable marker for a past EBV infection. In rare exceptional cases the IgG immune response against EBNA1 (primary or secondary) may not appear. In immunosuppressed patients IgG antibody titers against EBNA1 may greatly decrease (secondary EBNA1 loss).</td>
<td>IgG: central highly specific marker for past EBV infection</td>
</tr>
<tr>
<td>VCA</td>
<td>Several “Virus Capsid Antigens” have been described. Among them the proteins gp125 and p18 are regarded as immunodominant. As a rule IgM antibodies against VCA-gp125/p18 disappear again several weeks after infection, IgG antibodies against VCA-gp125/p18 are kept lifelong. Sometimes IgM antibodies against VCA-gp125/p18 are built again in reactivations.</td>
<td>IgG: highly specific general marker for EBV infections</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgM: highly specific for an EBV primary infection</td>
</tr>
<tr>
<td>EA-D</td>
<td>“Early Antigen-Diffuse” belongs to the early antigens, which are synthesised in the viral replication cycle (acute phase of infection). IgG and IgM antibodies against EA-D appear with negative EBNA1-IgG at the same time in primary infections typically. IgG antibodies against EA-D decrease during convalescence but may greatly increase again during EBV reactivations. But this antibody increase is no statement about clinical relevance of EBV reactivation.</td>
<td>IgG: 1.) specific for EBV primary infection</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.) serological marker for EBV reactivation</td>
</tr>
</tbody>
</table>

9.5 Interpretation Scheme

<table>
<thead>
<tr>
<th>Diagnosis group</th>
<th>IgM</th>
<th>IgG</th>
<th>Heterophile Antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>VCA</td>
</tr>
<tr>
<td>Seronegative</td>
<td>neg.</td>
<td>neg.</td>
<td>neg.</td>
</tr>
<tr>
<td>Primary Infection</td>
<td>pos./neg.</td>
<td>neg.</td>
<td>pos./neg.</td>
</tr>
<tr>
<td>Past Infection</td>
<td>neg.</td>
<td>pos./neg.</td>
<td>neg./pos.</td>
</tr>
</tbody>
</table>

pos./neg.: normally positive
neg./pos.: normally negative

Application of the diagnostic factor:
Under certain circumstances VCA IgM antibodies can persist in spite of an already existing EBNA1 IgG immune response. In such unclear cases when both highly specific markers are positive, i.e. EBNA1 IgG for previous infections and VCA IgM for primary infections, the diagnostic factor of 1.5 is an aid to clear diagnosis. The VU values of EBNA1 IgG and of VCA IgM are compared and the parameter, that is at least a factor of 1.5 greater than than the other is the one used for diagnosis.

If EBNA1 IgG and VCA IgM are both positive then:
- VU value for EBNA1 IgG \( \geq \) 1.5x VU value for VCA IgM \( \Rightarrow \) past infection
- VU value for VCA IgM \( \geq \) 1.5x VU value for EBNA1 IgG \( \Rightarrow \) primary infection
Example: VCA IgM: 25VE and EBNA1 IgG: 15VU

\[
\text{VU value for VCA IgM: 25VU} \geq 1.5 \times 15VU \quad \text{Wert for EBNA1 IgG: 22.5VU} \\
\text{VCA IgM: 25VU} \geq \text{EBNA1 IgG: 22.5VU} \quad \Rightarrow \text{primary infection}
\]

Since VCA IgM is at least a factor of 1.5 higher than EBNA1 IgG a clear diagnosis can be made of a primary infection.

9.6 Limits of the Test

1. The interpretation of serological results shall always include the clinical picture, epidemiological data and all further available laboratory results.
2. A negative ELISA result does not completely exclude an EBV infection.
3. Infective agents with similar clinical picture should be considered in differential diagnosis.
4. Crossreactivities of Epstein Barr Virus against the Herpes Virus family are known. With a positive IgM result specially crossreactivities against CMV should be excluded.
5. A negative IgM result does not exclude the possibility of a primary infection, as in some cases no IgM is built during an acute infection (IgM-nonresponder) (8,9).
6. At the beginning of a primary infection the serology of all parameters may be negative. With clinical suspicion of an EBV infection and a negative serology a second blood sample should be taken.
7. A negative anti-EBNA1 is not necessarily an indication of a primary infection. There can be a secondary anti-EBNA1 loss in immunosuppressed patients and no EBNA1 antibody is formed by 5% of those infected with EBV (EBNA1-non-responders) (8, 9).
8. An accurate interpretation of an EBV infection should be used on the results of the lead antigens VCA IgG, VCA IgM and EBNA1 IgG antibodies, using ELISA, Western Blot or Immunoblot. The EA-D IgG can provide additional information for primary infections and serological reactivations.
9. Antibodies which were passively transmitted shortly before examination may influence the EBV serological result. This might be e.g. by blood transfusion or maternally transmitted antibodies to the infant.
10. The EBV serology does not permit reliable conclusions to be made concerning the clinical relevance of a reactivation (11).

10. Performance Data

10.1 Diagnostic sensitivity

The following clinically characterized serum collective was investigated to determine the diagnostic sensitivity: sera from patients with EBV primary (n=51, sera from Dr. Gärtner, Homburg/Saar). Immunoblots and/or IFA were used as reference tests.

<table>
<thead>
<tr>
<th>Sera Collective (n=51)</th>
<th>VT EBV ELISA IgG + IgM overall result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Finding</td>
<td>Primary Infection</td>
</tr>
<tr>
<td>Primary Infection</td>
<td>51</td>
</tr>
<tr>
<td>Past Infection</td>
<td>0</td>
</tr>
</tbody>
</table>

In the overall evaluation the VT EBV ELISA results are in agreement with the prior clinical evaluations.

10.2 Analytical sensitivity

The following serum collectives were investigated to determine the analytical sensitivity:

1. patients with primary EBV infections (n=123; serum source: Dr. Gärtner, Homburg/Saar, serum panel of a commercial supplier, interlaboratory investigation sera, blood donor sera and routine sera).
2. past EBV infections (n=231; serum source: Dr. Gärtner, Homburg/Saar, interlaboratory investigation sera, serum panel of a commercial supplier, blood donor sera and routine sera).
Immunoblots and/or IFTs were carried out as references.

<table>
<thead>
<tr>
<th>Serum collective (n=354)</th>
<th>VT EBV ELISA IgG + IgM overall result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary infection</td>
</tr>
<tr>
<td>Analytical result</td>
<td></td>
</tr>
<tr>
<td>Primary infection</td>
<td>120</td>
</tr>
<tr>
<td>Past infection</td>
<td>0</td>
</tr>
</tbody>
</table>

In the overall evaluation there is complete agreement between the VT EBV ELISA results and the prior analytical results.

Unclear results cannot be taken into account for the analytical sensitivity.

10.3 Analytical specificity

The following serum collective was investigated to determine the analytical specificity:
Seronegative (n=22, serum source: Dr. Gärtner, Homburg/Saar, interlaboratory investigation sera), pregnancy sera (n=15), child sera (n=10) and routine sera (n=32). Immunoblots and/or IFTs were carried out as references.

In the overall evaluation there is complete agreement between the VT EBV ELISA results and the prior analytical results.

Unclear results cannot be taken into account for the analytical specificity.

(*) The unclear results were obtained with one child serum that did not yield an unequivocal serological ELISA result and again 4 sera yielded only a positive VCA IgG result in both ELISA and Immunoblot.

10.3 Diagnostic factor

Six sera yielding unclear results, with a result for both VCA IgM and EBNA1 IgG, were investigated to check the performance of the diagnostic factor.

<table>
<thead>
<tr>
<th>Serum No.</th>
<th>VCA IgM</th>
<th>EBNA1 IgG</th>
<th>Clear diagnosis using diagnostic factor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VU valueWert</td>
<td>Assessment</td>
<td>VU value</td>
</tr>
<tr>
<td>1</td>
<td>37.5</td>
<td>positive</td>
<td>11.7</td>
</tr>
<tr>
<td>2</td>
<td>25.0</td>
<td>positive</td>
<td>14.2</td>
</tr>
<tr>
<td>3</td>
<td>36.7</td>
<td>positive</td>
<td>65.6</td>
</tr>
<tr>
<td>4</td>
<td>20.7</td>
<td>positive</td>
<td>78.9</td>
</tr>
<tr>
<td>5</td>
<td>19.1</td>
<td>positive</td>
<td>54.8</td>
</tr>
<tr>
<td>6</td>
<td>21.1</td>
<td>positive</td>
<td>67.0</td>
</tr>
</tbody>
</table>

A clear diagnosis could be made with the aid of the diagnostic factor for all the sera investigated.
10.4 Prevalence (Expected Values)

80 sera were tested to determine the prevalence rate of 95% in adults (past EBV infection) as described in literature (10).

<table>
<thead>
<tr>
<th>Seronegatives</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Past Infections</td>
<td>76</td>
</tr>
<tr>
<td>Primary Infections</td>
<td>0</td>
</tr>
</tbody>
</table>

10.5 Intra-assay-Coefficient of Variation (Repeatability)

In one assay, strips of different plates of one batch have been tested with two sera. The obtained coefficient of variation is for:

- VCA IgG, EBNA1: < 9%
- VCA IgM, EA-D: < 15%

10.6 Inter-assay-Coefficient of Variation (Reproducibility)

Three sera were tested in 10 independent test runs by different persons in different laboratories. The obtained coefficient of variation is for:

- VCA IgG, VCA IgM, EBNA1, EA-D: < 20%

11. Literature

12. Test Procedure Scheme

Preparation of Patient Samples and Washing Solution

▼ Washing Solution: Fill up concentrate to 1 liter with aqua dest./demin.

▼ IgG-Samples – Dilution
1:101

IgM-Samples - Dilution
1:101

Rheumafactor-absorption with RF-SorboTech

e.g.:
10 µl serum/plasma + 1000 µl Dilution Buffer
(Serum Dilution Buffer is ready to use)

e.g.:
5 µl serum/plasma + 450 µl Dilution Buffer +
1 drop RF-SorboTech, incubate for 15 min. at room temperature.

Testprocedure

Samples Incubation
30 minutes at 37°C

100 µl Patient Samples
blank value (Dilution Buffer) and controls

Wash 4times

400 µl Washing Solution
Remove Residues on a Cellulose Pad

Conjugate Incubation
30 minutes at 37°C

100 µl Conjugate
IgG, IgM

Wash 4times

400 µl Washing Solution
Remove Residues on a Cellulose Pad

Substrate Incubation
30 minutes at 37°C

100 µl Substrate

Stopping

50 µl Stopping Solution
shake carefully

Measure Extinctions

Photometer at 450/620nm
(Reference Wavelength 620-690nm)